Citrullinated α-enolase as a novel target for cancer immunotherapy

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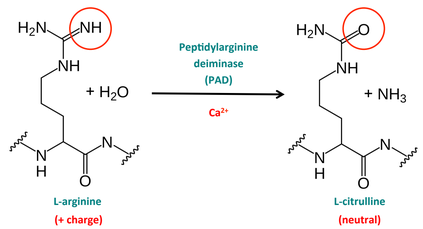
INTRODUCTION

- CD4 cells are potent effectors but CD4 responses to self antigens are attenuated.
- Cellular stress induces autophagy which leads to modification of proteins recognised by the immune system (1).
- In the absence of inflammation, immunity is regulated, whereas in its presence CD4 responses to modified self-antigens are stimulated (2).
- T cells targeting modified self-antigens play a role in the pathophysiology of several autoimmune diseases.
- Cancer cells citrullinate proteins (3) and present modified peptides as targets for CD4 T cells (4).



Citrullination. A modification that occurs within stressed cells. Peptidylarginine deiminase (PADs) enzymes are activated and convert arginine to citrulline by altering the positively charge aldimine group (=NH) group of arginine to the neutrally charged ketone group (=O) of citrulline.

Enolase

Alpha enolase is a glycolytic protein which is upregulated in many cancers and is known to undergo citrullination. Screening of Enolase peptides identified citrullinated (cit) peptides which induce immune responses that do not cross react with equivalent wild type (wt) peptide.

Citrullinated α-enolase peptides induce IFNγ responses in mice

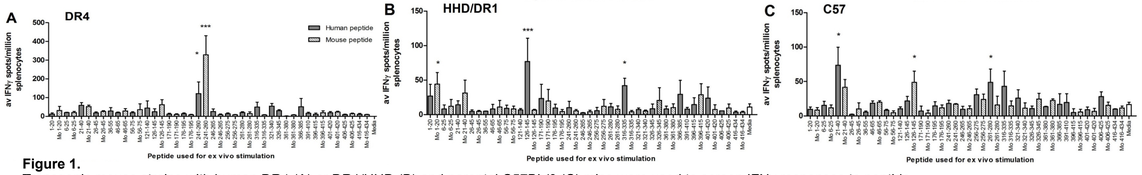


Figure 1.
Transgenic mouse strains with human DR4 (A) or DR1/HHD (B) and parental C57BL/6 (C) mice were used to screen IFNγ responses to peptide.
Mice were immunised with pools of 4-6 non-overlapping Human citrullinated Enolase peptides. 14 days after *Ex vivo* responses to stimulation with Human and mouse equivalent peptides were assessed by IFNγ ELISpot.

Citrullinated α-enolase responses are detected in healthy donors and cancer patients

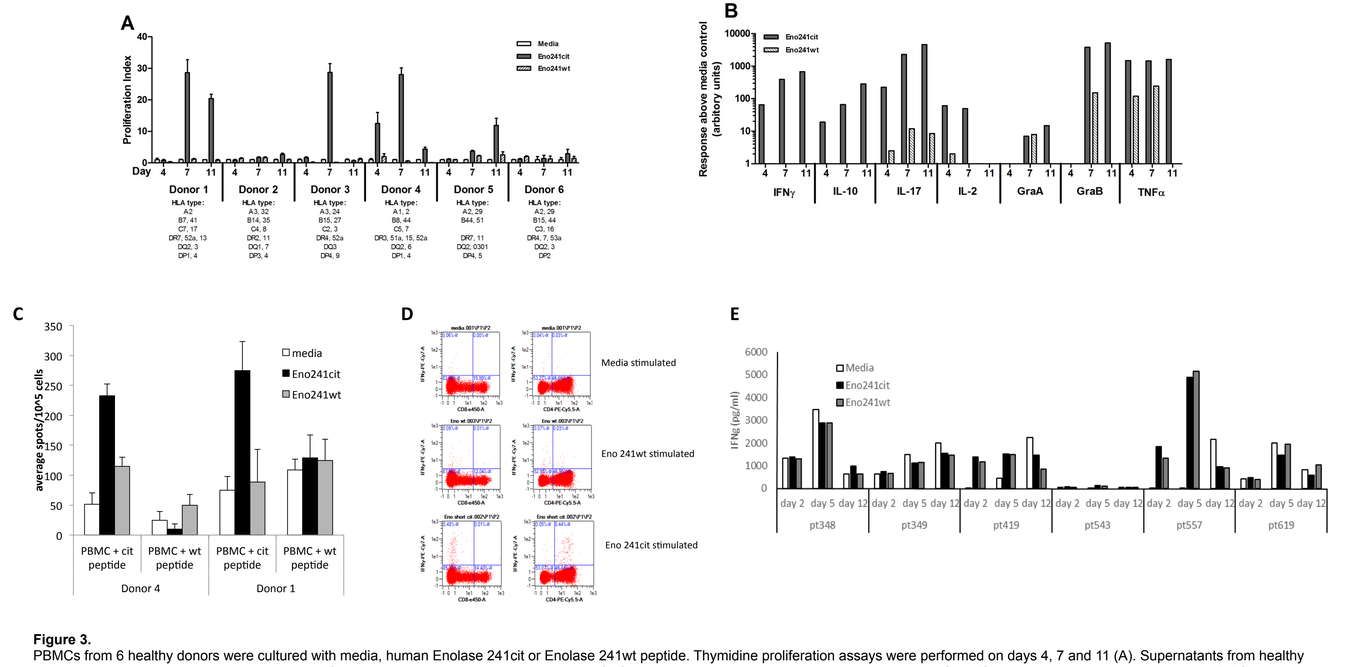


Figure 3.
PBMCs from 6 healthy donors were cultured with media, human Enolase 241cit or Enolase 241wt peptide. Thymidine proliferation assays were performed on days 4, 7 and 11 (A). Supernatants from healthy donor 4 on day 11 were collected and analysed for cytokine levels using Luminex (B). PBMCs from healthy donors 1 and 4 were tested in IFNγ ELISpot (C) or by intracellular cytokine staining (D) for peptide specific IFNγ responses at day 12 of culture. PBMCs from 6 Ovarian/TNB cancer patients were cultured with media, human Enolase 241cit or Enolase 241wt peptide. Supernatants were collected at days 2, 5 and 12 and analysed for cytokine levels using Luminex (E)

Responses in mice are CD4 mediated and show cytotoxicity

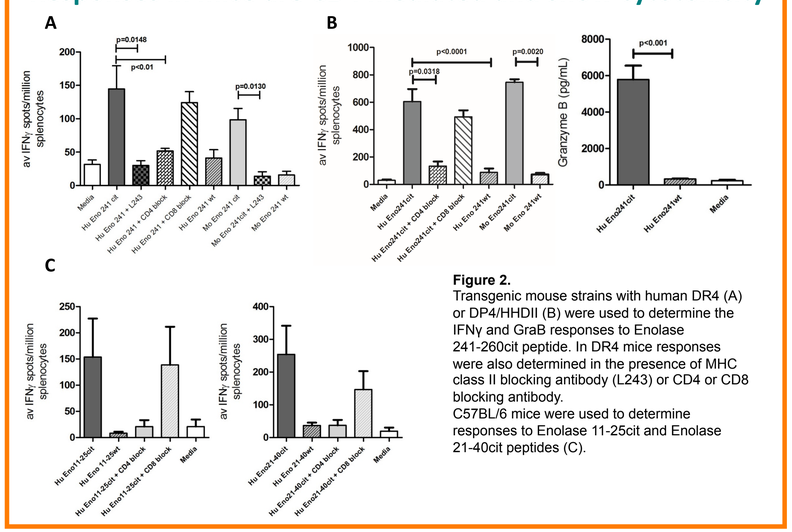


Figure 2.
Transgenic mouse strains with human DR4 (A) or DP4/HHDII (B) were used to determine the IFNγ and GraB responses to Enolase 241-260cit peptide. In DR4 mice responses were also determined in the presence of MHC class II blocking antibody (L243) or CD4 or CD8 blocking antibody.
C57BL/6 mice were used to determine responses to Enolase 11-25cit and Enolase 21-40cit peptides (C).

Citrullinated α-enolase peptide immunisation provides efficient tumour therapy

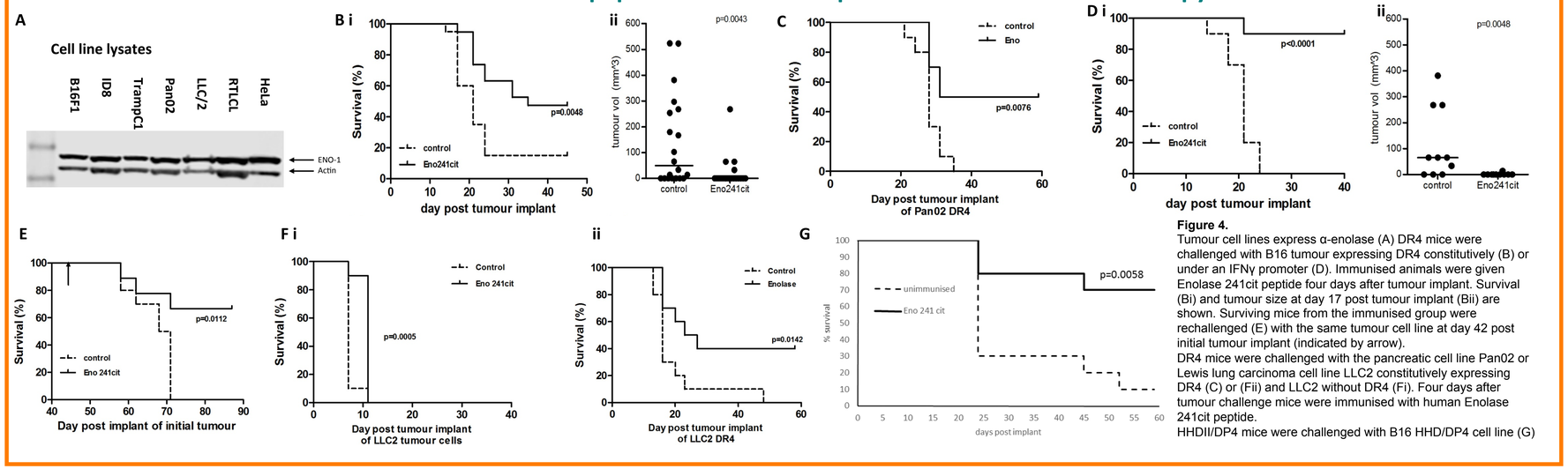


Figure 4.
Tumour cell lines express α-enolase (A) DR4 mice were challenged with B16 tumour expressing DR4 constitutively (B) or under an IFNγ promoter (D). Immunised animals were given Enolase 241cit peptide four days after tumour implant. Survival (Bi) and tumour size at day 17 post tumour implant (Bii) are shown. Surviving mice from the immunised group were rechallenged (E) with the same tumour cell line at day 42 post initial tumour implant (indicated by arrow).
DR4 mice were challenged with the pancreatic cell line Pan02 or Lewis lung carcinoma cell line LLC2 constitutively expressing DR4 (C) or (Fii) and LLC2 without DR4 (Fi). Four days after tumour challenge mice were immunised with human Enolase 241cit peptide.
HHDII/DP4 mice were challenged with B16 HHD/DP4 cell line (G)

CONCLUSION

- **Citrullinated Enolase peptides induce T cell responses in HLA transgenic and conventional mice**
- **The immune responses are CD4 mediated and specific to the citrullinated peptides**
- **Healthy donor PBMCs showed CD4 proliferative Th1 responses to Enolase 241cit peptide after in vitro culture**
- **Immunisation of HLA transgenic mice with citrullinated peptide was associated with increased survival in a number of tumour models**
- **Anti-tumour responses are dependent upon expression of MHC class II**

References:

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